UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/540,406	06/24/2005	Frank Bergmann	21581-US	8359

22829 7590 07/16/2007

ROCHE MOLECULAR SYSTEMS INC
PATENT LAW DEPARTMENT
1145 ATLANTIC AVENUE
ALAMEDA, CA 94501

EXAMINER	
THOMAS, DAVID C	

ART UNIT	PAPER NUMBER
1637	

MAIL DATE	DELIVERY MODE
07/16/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/540,406	BERGMANN ET AL.	
	Examiner	Art Unit	
	David C. Thomas	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 March 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-7* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-7* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's amendment filed March 16, 2007 is acknowledged. Claim 1 (currently amended) and claims 2-7 (previously amended) will be examined on the merits. Claims 11-14 were previously withdrawn and have now been canceled. Claims 8-10 were previously canceled.

Claim Rejections - 35 USC § 103

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

4. Claims 1-3, 5 and 6 are rejected under 35 U.S.C. 103(a) as being anticipated by Grunau et al. (Nucleic Acids Res. (2001) 29: e65, 1-7).

Grunau teaches a method for the conversion of a cytosine base in a nucleic acid to an uracil base (for overview, see Abstract and p. 3, column 2, lines 21-28) comprising:

a) incubating a solution comprising the nucleic acid for a time period of 1.5 to 3.5 hours at a temperature between 70 and 90°C, wherein the concentration of bisulfite in the solution is between 3 M and 6.25 M and wherein the pH value of the solution is between 5.0 and 6.0, whereby the nucleic acid is deaminated (deamination of the DNA was achieved by treating alkaline denatured DNA with either of two bisulfite solutions, one at between 3.87-4.26 M bisulfite and the other at 5.2-5.69 M bisulfite, at temperatures in the range of 0-90°C including 80 and 85°C and at a final pH adjusted to 5.0, and for periods of either 1 hour or 4 hours, such that the DNA was exposed to bisulfite at the latter time for 1.5 to 3.5 hours during the 4-hour incubation, p. 2, column 1, line 33 to column 2, line 26 and Table 1), and

b) incubating the solution comprising the deaminated nucleic acid under alkaline conditions whereby the deaminated nucleic acid is desulfonated (the DNA was desulfonated by addition of 3M NaOH solution to a buffered DNA solution at pH 8, p. 2, column 2, lines 27-35).

With regard to claim 2, Grunau teaches a method wherein in step a) the temperature is between 75 and 85°C (the DNA is treated at 80 or 85°C during the deamination procedure, p. 2, column 2, lines 20-26 and Table 1).

With regard to claim 3, Grunau teaches a method wherein the concentration of bisulfite is between 3.2 M and 6 M (either of two bisulfite solutions is used for

deamination, one at between 3.87-4.26 M bisulfite and the other at 5.2-5.69 M bisulfite, p. 2, column 1, line 33 to column 2, line 1 and column 2, lines 17-19).

With regard to claims 5 and 6, Grunau teaches a method wherein the time period is between 1.75 and 3 hours, or between 2 and 3 hours (the time period of incubation is either 1 or 4 hours, such that the DNA was exposed to bisulfite at the latter time for 1.5 to 3.5 hours or 2 to 3 hours during the 4-hour incubation, p. 2, column 2, lines 20-26 and Table 1).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to optimize the methods of Grunau for deamination of a cytosine base to a uracil base in a nucleic acid for a time period that is not less than 1.5 hours but not more than 3.5 hours since Grunau teaches time periods of bisulfite treatment for 1 hour at 95°C and 4 hours at 55°C under similar conditions of reagent concentrations that efficiently convert all cytosines to uracil (Grunau, p. 6, column 1, lines 12-16). Thus, an ordinary practitioner would have been motivated to optimize the methods of Grunau for efficient conversion of a cytosine base to a uracil base in a nucleic acid for a time period that is not less than 1.5 hours but not more than 3.5 hours since it would require only routine optimization of reaction conditions within this time range that is known to be efficient for cytosine deamination (see Table 2). This is consistent with the Federal Circuit decision in In re Peterson, 65 USPQ2d 1379, 1382 (Fed. Cir. 2003) "We have also held that a prima facie case of obviousness exists when the claimed range and the prior art range do not overlap but are close enough such that one skilled in the art would have expected them to have the same properties." Thus, an

ordinary practitioner would have recognized from the results that the pH could be adjusted to maximize the desired results. As noted in *In re Aller*, 105 USPQ 233 at 235,

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of specific incubation times was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art. As noted, a skilled artisan would expect incubation times of 1 hour and 4 hours and values in between to have similar properties in the deamination of cytosine bases to uracil bases in DNA when concomitantly modifying the incubation temperature. Thus, an ordinary practitioner would have recognized that the results could be adjusted to maximize the desired results.

5. Claim 4 is rejected under 35 U.S.C. 103(a) as being unpatentable over Grunau et al. (Nucleic Acids Res. (2001) 29: e65, 1-7) in view of Hayatsu et al. (Biochemistry (1970) 9: 2858-2865).

Grunau teaches the limitations of claims 1-3, 5 and 6 as discussed above.

Grunau does not teach a method wherein the pH value of the solution is between 5.25 and 5.75.

With regard to claim 4, Hayatsu teaches a method of deamination of cytosine to uracil in 3M bisulfite solutions at pH values between 4 and 6.5 (p. 2862, column 1, line 31 to column 2, line 11).

Hayatsu does not teach a method of deamination of a cytosine base to a uracil base in a nucleic acid at temperatures between 70 and 90°C, for time periods of 1.5 to 3.5 hours.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to combine the methods of Grunau and Hayatsu for deamination of a cytosine base to a uracil base in a nucleic acid at a pH of between 5.25 and 5.75 since both references teach that efficient conversion takes place at either pH 5 or pH 6 (see Grunau, Table 1 for pH 5 and Hayatsu, p. 2862, column 2, lines 1-4 for pH 5-6) under similar conditions of reagent concentrations, as well as time of exposure to the bisulfite reagent. Thus, an ordinary practitioner would have been motivated to combine the methods of Grunau and Hayatsu for efficient conversion of a cytosine base to a uracil base in a nucleic acid at a pH of 5.5 since it would require only routine optimization of reaction conditions in this narrow pH range that are known to be efficient for cytosine deamination. This is consistent with the Federal Circuit decision in In re Peterson, 65 USPQ2d 1379, 1382 (Fed. Cir. 2003) "We have also held that a prima facie case of obviousness exists when the claimed range and the prior art range do not overlap but are close enough such that one skilled in the art would have expected them to have the same properties." Thus, an ordinary practitioner would have recognized from the results that the pH could be adjusted to maximize the desired results. As noted in *In re Aller*, 105 USPQ 233 at 235,

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of specific pH was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art. As noted, a skilled artisan would expect pH values of 5.0, 6.0 and values in between to have identical properties in the deamination of cytosine bases to uracil bases in DNA. Thus, an ordinary practitioner would have recognized that the results could be adjusted to maximize the desired results.

6. Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Grunau et al. (Nucleic Acids Res. (2001) 29: e65, 1-7) in view of Hayatsu et al. (Biochemistry (1970) 9: 2858-2865) and further in view of Olek et al. (Nucleic Acids Res. (1996) 24: 5064-5066).

Grunau teaches the limitations of claims 1-3, 5 and 6 as discussed above.

With regard to claim 7, Grunau teaches a method wherein in step a) the temperature is 80°C (the DNA is treated at 80 or 85°C during the deamination procedure, p. 2, column 2, lines 20-26 and Table 1) and the time period is between 2 and 3 hours (the time period of incubation is either 1 or 4 hours, such that the DNA was exposed to bisulfite at the latter time for 2 to 3 hours during the 4-hour incubation, p. 2, column 2, lines 20-26 and Table 1).

Grunau does not teach a method wherein the pH value of the solution is 5.5 and the concentration of the bisulfite solution is 5M.

Hayatsu teaches a method of deamination of cytosine to uracil in 3M bisulfite solutions at pH values between 4 and 6.5 (p. 2862, column 1, line 31 to column 2, line 11).

Hayatsu does not teach a method of deamination of a cytosine base to a uracil base in a nucleic acid at 80°C in a 5M bisulfite solution for time periods of 2 to 3 hours.

With regard to claim 7, Olek teaches a method of bisulfite treatment of chromosomal DNA to convert cytosine bases to uracil bases using a solution of 5M bisulfite (p. 5065, column 1, lines 6-11) at pH 5, and treating for 4 hours at 50°C.

Olek does not teach a method of deamination of a cytosine base to a uracil base in a nucleic acid at 80°C at a pH of 5.5 for time periods of 2 to 3 hours.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to combine the methods of Grunau, Hayatsu and Olek for deamination of a cytosine base to a uracil base in a nucleic acid at a pH of 5.5, a temperature of 80°C, a bisulfite concentration of 5 M, and exposure to bisulfite for 2-3 hours since all three references teach that efficient conversion takes place at pH values in the range of 5-6 as well as under similar conditions of temperature, reagent concentrations, and reaction times (see Grunau, Table 1 for pH 5, Hayatsu, p. 2862, column 2, lines 1-4 for pH 5-6, and Olek, p. 5065, column 1, lines 6-11). Thus, an ordinary practitioner would have been motivated to combine the methods of Grunau, Hayatsu and Olek for efficient conversion of a cytosine base to a uracil base in a nucleic acid since it would require only routine optimization of reaction conditions in the pH, temperature, bisulfite concentration, and exposure time ranges that are known to be

efficient for cytosine deamination. This is consistent with the Federal Circuit decision in In re Peterson, 65 USPQ2d 1379, 1382 (Fed. Cir. 2003) "We have also held that a prima facie case of obviousness exists when the claimed range and the prior art range do not overlap but are close enough such that one skilled in the art would have expected them to have the same properties." Thus, an ordinary practitioner would have recognized from the results that the pH, temperature, reagent concentration, and reaction time could be adjusted to maximize the desired results. As noted in *In re Aller*, 105 USPQ 233 at 235,

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of specific reaction conditions was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art. As noted, a skilled artisan would expect pH values of 5.0, 6.0 and values in between to have identical properties in the deamination of cytosine bases to uracil bases in DNA, as well as bisulfite concentrations in the range of 4-6 M, temperatures between 50 and 80°C, and varying reaction times up to 4 hours. Thus, an ordinary practitioner would have recognized that the results could be adjusted to maximize the desired results.

Response to Arguments

7. Applicant's arguments filed March 16, 2007 have been fully considered but they are not persuasive.

Claims 1-7 have been corrected to include an appropriate opening phrase and therefore the claim objection has been withdrawn.

Applicant argues that the rejection of claims 1-3, 5 and 6 under U.S.C. § 102(b) as being anticipated by Grunau et al. (Nucleic Acids Res. (2001) 29:e65, 1-7) should be withdrawn since the reference no longer teaches all the limitations of the claims as amended. In particular, Applicant argues that Grunau does not disclose incubating a solution comprising the nucleic acid and bisulfite for a time period that is not less than 1.5 hours but not more than 3.5 hours since the time periods for incubation taught by Grunau are either one hour or four hours. The Examiner agrees that Grunau does not teach a method wherein the incubation time is for a time period within the range of 1.5 to 3.5 hours, but not exceeding 3.5 hours and therefore the 102(b) rejection is withdrawn.

However, since the time period taught by Grunau falls within the range of one to four hours that has been shown to be highly efficient for cytosine deamination (see Table 2 and Grunau, p. 6, column 1, lines 12-16), it would be obvious to one of ordinary skill in the art that efficient deamination can be achieved by reducing the incubation time from four hours while at the same time increasing the incubation temperature from 55°C, or conversely, increasing the incubation time from one hour while at the same time decreasing the incubation temperature from 95°C. In fact, Grunau teaches that "5mC is not deaminated at 95°C and as far as this fact is concerned every temperature

above 55°C can be used" (p. 5, column 2, lines 17-19) suggesting that optimization of incubation time and temperature are possible, with the understanding that degradation of the DNA occurs much faster at 95°C and therefore this range should only be used when sufficient amounts of DNA are available (p. 5, column 2, lines 13-17). Thus, Grunau provides both the necessary parameters and motivation for one of ordinary skill in the art to optimize conditions for bisulfite treatment, since the incubation time could be shortened from four hours to save time while also maintaining the integrity of the DNA by using incubation temperatures lower than 95°C. Therefore, claims 1-3, 5 and 6 are rejected under U.S.C. § 103(a) as being unpatentable over Grunau.

Applicant then argues that the rejection of claims 4 and 7 under 35 U.S.C. § 103(a) as being unpatentable over Grunau in view of Hayatsu et al. (Biochemistry (1970) 9: 2858-2865) and Grunau in view of Hayatsu and further in view of Olek et al. (Nucleic Acids Res. (1996) 24:5064-5066), respectively, should be withdrawn since the combination of these references no longer teach all the limitations of the claims as amended. In particular, Applicant argues that none of the references teach incubating a solution comprising the nucleic acid and bisulfite for a time period that is not less than 1.5 hours but not more than 3.5 hours. As discussed above, it would be obvious to one of ordinary skill in the art to optimize the incubation conditions based on the time and temperature ranges taught by Grunau for efficient deamination of cytosine. Since the secondary references teach the required limitations of pH values and bisulfite concentrations, both 103 rejections are maintained.

Finally, Applicant argues that there is no motivation to optimize the conditions taught by Grunau since this reference states that optimization was already performed to provide "a comprehensive investigation of the influence of time and temperature of the bisulfite reaction on the sensitivity and specificity of the bisulfite sequencing method and deliver an estimation of the degree of DNA degradation during the treatment (p. 1, column 2, line 39 to p. 2, column 1, line 2). As discussed above, Grunau teaches incubation times of one and four hours at 95°C and 55°C, respectively. However, the reference also suggests that any temperature above 55°C can be used and, given that temperatures in the range of 95°C cause higher levels of DNA degradation, one of ordinary skill in the art would realize that the incubation time must be reduced as the temperature is increased to prevent degradation. Since the time parameters taught by Grunau flank the time range cited as a limitation in claim 1 (1.5-3.5 hours), optimization that involves increasing or decreasing the incubation time in conjunction with varying the temperature will necessarily overlap this range.

Summary

8. Claims 1-7 are rejected. No claims are allowable.

Conclusion

9. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Correspondence

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to David C. Thomas whose telephone number is 571-272-3320 and whose fax number is 571-273-3320. The examiner can normally be reached on 5 days, 9-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

David C. Thomas 7/12/07

David C. Thomas
Patent Examiner
Art Unit 1637

JEFFREY FREDMAN
PRIMARY EXAMINER
7/12/07